

CLAIMS

1. A method for detecting allergens by using 2 or more monoclonal antibodies recognizing native and denatured milk allergens, native and denatured albumen allergens, native and denatured flour allergens, native and denatured buckwheat allergens, or native and denatured peanut allergens, using $\alpha s1\alpha s1$ casein which is the main protein of $\alpha s1$ casein, β -lactoglobulin which is the main protein of whey, ovalbumin and ovomucoid which are main proteins of albumen, gliadin which is the main protein of flour, proteins with a molecular weight of 24kDa and 76kDa which are main proteins of buckwheat, or Arah1 which is the main protein of peanut, as an index.

2. A method for detecting milk allergens wherein a monoclonal antibody recognizing native milk allergens and a monoclonal antibody recognizing denatured milk allergens are used in combination.

3. The method for detecting milk allergens according to claim 2, wherein 2 or more monoclonal antibodies recognizing different epitopes are used as monoclonal antibodies recognizing native milk allergens and/or denatured milk allergens.

4. The method for detecting milk allergens according to claim 2 or 3, wherein the monoclonal antibody recognizing native milk allergens and/or denatured milk allergens is

an anti- α s1 casein monoclonal antibody.

5. The method for detecting milk allergens according to claim 4, wherein the anti- α s1 casein monoclonal antibody recognizes a native α s1 casein, an urea-treated α s1 casein, a native sodium casein and a denatured sodium casein.

6. The method for detecting milk allergens according to claim 4 or 5, wherein the anti- α s1 casein monoclonal antibody recognizes the 132 - 193 position of the amino acid sequence of α s1 casein shown by SEQ ID NO:1.

7. The method for detecting milk allergens according to any one of claims 4 to 6, wherein the anti- α s1 casein monoclonal antibody is the anti- α s1 casein monoclonal antibody Pas1CN1 produced by hybridoma (FERM ABP-10263) and/or the anti- α s1 casein monoclonal antibody Pas1CN2 produced by hybridoma (FERM ABP-10264).

8. The method for detecting milk allergens according to any one of claims 4 to 7, wherein the native α s1 casein and the urea-treated α s1 casein in foods can be analyzed qualitatively and quantitatively even at a concentration in a range of 10 to 1000 ppb by sandwich ELISA.

9. The method for detecting milk allergens according to claim 2 or 3, wherein the monoclonal antibody recognizing native milk allergens and/or denatured milk allergens is an anti- β -lactoglobulin monoclonal antibody.

10. The method for detecting milk allergens according to claim 9, wherein the anti- β -lactoglobulin monoclonal antibody recognizes a native β -lactoglobulin, an urea-treated β -lactoglobulin, and a reduced-carboxymethylated β -lactoglobulin.

11. The method for detecting milk allergens according to claim 9 or 10, wherein the anti- β -lactoglobulin monoclonal antibody is the anti- β -lactoglobulin monoclonal antibody P β GL1 produced by hybridoma (FERM ABP-10281) and/or the anti- β -lactoglobulin monoclonal antibody P β GL2 produced by hybridoma (FERM ABP-10282) and/or the anti- β -lactoglobulin monoclonal antibody P β GL3 produced by hybridoma (FERM ABP-10283).

12. The method for detecting milk allergens according to any one of claims 9 to 11, wherein the native β -lactoglobulin and the urea-treated β -lactoglobulin in foods can be analyzed qualitatively and quantitatively even at a concentration in a range of 30 to 1000 ppb by sandwich ELISA.

13. The method for detecting milk allergens according to any one of claims 2 to 12, wherein a casein and/or a whey protein is extracted with the use of urea and 2-mercaptoethanol from a sample.

14. The method for detecting milk allergens according to

any one of claims 1 to 13, wherein 1 or more monoclonal antibodies recognizing a native casein and 1 or more monoclonal antibodies recognizing a denatured casein and 1 or more monoclonal antibodies recognizing a native β -lactoglobulin and 1 or more monoclonal antibodies recognizing a denatured β -lactoglobulin are used.

15. A kit for detecting milk allergens comprising a monoclonal antibody recognizing native milk allergens and a monoclonal antibody recognizing denatured milk allergens, which is used under a condition that a monoclonal antibody recognizing native milk allergens and a monoclonal antibody recognizing denatured milk allergens are used in combination.

16. The kit for detecting milk allergens according to claim 15, comprising 2 or more monoclonal antibodies recognizing different epitopes as monoclonal antibodies recognizing native milk allergens and/or denatured milk allergens.

17. The kit for detecting milk allergens according to claim 15 or 16, wherein the monoclonal antibody recognizing native milk allergens and/or denatured milk allergens is an anti- α s1 casein monoclonal antibody.

18. The kit for detecting milk allergens according to claim 17, wherein the anti- α s1 casein monoclonal antibody recognizes a native α s1 casein, an urea-treated α s1 casein,

a native sodium casein, and a denatured sodium casein.

19. The kit for detecting milk allergens according to claim 17 or 18, wherein the anti- α s1 casein monoclonal antibody recognizes the 132 - 193 position of the amino acid sequence of α s1 casein shown by SEQ ID NO:1.

20. The kit for detecting milk allergens according to any one of claims 17 to 19, wherein the anti- α s1 casein monoclonal antibody is the anti- α s1 casein monoclonal antibody Pas1CN1 produced by hybridoma (FERM ABP-10263) and/or the anti- α s1 casein monoclonal antibody Pas1CN2 produced by hybridoma (FERM ABP-10264).

21. The kit for detecting milk allergens according to claim 15 or 16, wherein the monoclonal antibody recognizing native milk allergens and/or denatured milk allergens is an anti- β -lactoglobulin antibody.

22. The kit for detecting milk allergens according to claim 21, wherein the anti- β -lactoglobulin monoclonal antibody recognizes a native β -lactoglobulin, an urea-treated β -lactoglobulin, and a reduced-carboxymethylated β -lactoglobulin.

23. The kit for detecting milk allergens according to claim 21 or 22, wherein the anti- β -lactoglobulin monoclonal antibody is the anti- β -lactoglobulin monoclonal antibody P β LG1 produced by hybridoma (FERM

ABP-10281) and/or the anti- β -lactoglobulin monoclonal antibody P β LG2 produced by hybridoma (FERM ABP-10282), and/or the anti- β -lactoglobulin monoclonal antibody P β LG3 produced by hybridoma (FERM ABP-10283).

24. The kit for detecting milk allergens according to any one of claims 15 to 23, wherein at least one of the 2 types of monoclonal antibodies recognizing different epitopes is a monoclonal antibody labeled with gold colloid used for immunochromatography.

25. The kit for detecting milk allergens according to claims 15 to 24, comprising 1 or more monoclonal antibodies recognizing a native casein and 1 or more monoclonal antibodies recognizing a denatured casein, and 1 or more monoclonal antibodies recognizing a native β -lactoglobulin and 1 or more monoclonal antibodies recognizing a denatured β -lactoglobulin.

26. Anti- α s1 casein monoclonal antibody Pas1CN1 produced by hybridoma (FERM ABP-10263).

27. Anti- α s1 casein monoclonal antibody Pas1CN2 produced by hybridoma (FERM ABP-10264).

28. Anti- β -lactoglobulin monoclonal antibody P β LG1 produced by hybridoma (FERM ABP-10281).

29. Anti- β -lactoglobulin monoclonal antibody P β LG2

produced by hybridoma (FERM ABP-10282).

30. Anti- β -lactoglobulin monoclonal antibody P β LG3 produced by hybridoma (FERM ABP-10283).

31. A method for detecting albumen allergens, wherein a monoclonal antibody recognizing native albumen allergens and a monoclonal antibody recognizing denatured albumen allergens are used in combination.

32. The method for detecting albumen allergens according to claim 31, wherein 2 or more monoclonal antibodies recognizing different epitopes are used as monoclonal antibodies recognizing native albumen allergens and/or denatured albumen allergens.

33. The method for detecting albumen allergens according to claim 31 or 32, wherein the monoclonal antibody recognizing native albumen allergens and/or denatured albumen allergens is an anti-ovalbumin monoclonal antibody.

34. The method for detecting albumen allergens according to claim 33, wherein the anti-ovalbumin monoclonal antibody recognizes a native ovalbumin and/or a reduced-carboxymethylated ovalbumin.

35. The method for detecting albumen allergens according to claim 33 or 34, wherein the anti-ovalbumin monoclonal

antibody is the anti-ovalbumin monoclonal antibody PNOA1 produced by hybridoma (FERM ABP-10265), and/or the anti-ovalbumin monoclonal antibody PNOA2 produced by hybridoma (FERM ABP-10266), and/or the anti-ovalbumin monoclonal antibody PDOA1 produced by hybridoma (FERM ABP-10275), and/or the anti-ovalbumin monoclonal antibody PDOA2 produced by hybridoma (FERM ABP-10276).

36. The method for detecting albumen allergens according to any one of claims 33 to 35, wherein the native ovalbumin and/or the denatured ovalbumin in foods can be analyzed qualitatively and quantitatively even at a concentration in a range of 1.0 to 10.0 ppb by sandwich ELISA.

37. The method for detecting albumen allergens according to claim 31 or 32, wherein the monoclonal antibody recognizing native albumen allergens and/or denatured albumen allergens is an anti-ovomucoid monoclonal antibody.

38. The method for detecting albumen allergens according to claim 37, wherein the anti-ovomucoid monoclonal antibody recognizes a native ovomucoid and/or an urea-denatured ovomucoid.

39. The method for detecting albumen allergens according to claim 37 or 38, wherein the anti-ovomucoid monoclonal antibody is the anti-ovomucoid monoclonal antibody PNOM1 produced by hybridoma (FERM ABP-10279) and/or the

anti-ovomucoid monoclonal antibody PNOM2 produced by hybridoma (FERM ABP-10280) and/or the anti-ovomucoid monoclonal antibody PDOM1 produced by hybridoma (FERM ABP-10277) and/or the anti-ovomucoid monoclonal antibody PDOM2 produced by hybridoma (FERM ABP-10278).

40. The method for detecting albumen allergens according to any one of claims 34 to 36, wherein the native ovomucoid and/or the denatured ovomucoid in foods can be analyzed qualitatively and quantitatively even at a concentration in a range of 10 to 100 ppb by sandwich ELISA.

41. The method for detecting albumen allergens according to any one of claims 31 to 40, wherein an ovalbumin and/or an ovomucoid is extracted with the use of urea and 2-mercaptoethanol from a test substance.

42. The method for detecting albumen allergens according to any one of claims 31 to 41, wherein 1 or more monoclonal antibodies recognizing a native ovalbumin and 1 or more monoclonal antibodies recognizing a denatured ovalbumin, and 1 or more monoclonal antibodies recognizing a native ovomucoid and 1 or more monoclonal antibodies recognizing a denatured ovomucoid are used.

43. A kit for detecting albumen allergens comprising a monoclonal antibody recognizing native albumen allergens and a monoclonal antibody recognizing denatured albumen allergens, which is used under a condition that a

monoclonal antibody recognizing native albumen allergens and a monoclonal antibody recognizing denatured albumen allergens are used in combination.

44. The kit for detecting albumen allergens according to claim 43, comprising 2 or more monoclonal antibodies recognizing different epitopes as monoclonal antibodies recognizing native albumen allergens and/or denatured albumen allergens.

45. The kit for detecting albumen allergens according to claim 43 or 44, wherein the monoclonal antibody recognizing native albumen allergens and/or denatured albumen allergens is an anti-ovalbumin monoclonal antibody.

46. The kit for detecting albumen allergens according to claim 45, wherein the anti-ovalbumin monoclonal antibody recognizes a native ovalbumin and/or a reduced carboxymethylated ovalbumin.

47. The kit for detecting albumen allergens according to claim 45 or 46, wherein the anti-ovalbumin monoclonal antibody is the anti-ovalbumin monoclonal antibody PNOA1 produced by hybridoma (FERM ABP-10265) and/or the anti-ovalbumin monoclonal antibody PNOA2 produced by hybridoma (FERM ABP-10266) and/or the anti-ovalbumin monoclonal antibody PDOA1 produced by hybridoma (FERM ABP-10275) and/or the anti-ovalbumin monoclonal antibody

PDOA2 produced by hybridoma (FERM ABP-10276).

48. The kit for detecting albumen allergens according to claim 439 or 44, wherein the monoclonal antibody recognizing native albumen allergens and/or denatured albumen allergens is an anti-ovomucoid monoclonal antibody.

49. The kit for detecting albumen allergens according to claim 48, wherein the anti-ovomucoid monoclonal antibody recognizes a native ovomucoid and/or an urea-denatured ovomucoid.

50. The kit for detecting albumen allergens according to claim 48 or 49, wherein the anti-ovomucoid monoclonal antibody is the anti-ovomucoid monoclonal antibody PNOM1 produced by hybridoma (FERM ABP-10279) and/or the anti-ovomucoid monoclonal antibody PNOM2 produced by hybridoma (FERM ABP-10280) and/or the anti-ovomucoid monoclonal antibody PDOM1 produced by hybridoma (FERM ABP-10277) and/or the anti-ovomucoid monoclonal antibody PDOM2 produced by hybridoma (FERM ABP-10278).

51. The kit for detecting albumen allergens according to any one of claims 43 to 50, wherein at least one of the 2 monoclonal antibodies recognizing different epitopes is a monoclonal antibody labeled with gold colloid used for immunochromatography.

52. The kit for detecting albumen allergens according to any one of claims 43 to 51, comprising 1 or more monoclonal antibodies recognizing a native ovalbumin and 1 or more monoclonal antibodies recognizing a denatured ovalbumin, and 1 or more monoclonal antibodies recognizing a native ovomucoid and 1 or more monoclonal antibodies recognizing a denatured ovomucoid.

53. Anti-ovalbumin monoclonal antibody PNOA1 produced by hybridoma (FERM ABP-10265).

54. Anti-ovalbumin monoclonal antibody PNOA2 produced by hybridoma (FERM ABP-10266).

55. Anti-ovalbumin monoclonal antibody PDOA1 produced by hybridoma (FERM ABP-10275).

56. Anti-ovalbumin monoclonal antibody PDOA2 produced by hybridoma (FERM ABP-10276).

57. Anti-ovomucoid monoclonal antibody PNOM1 produced by hybridoma (FERM ABP-10279).

58. Anti-ovomucoid monoclonal antibody PNOM2 produced by hybridoma (FERM ABP-10280).

59. Anti-ovomucoid monoclonal antibody PDOM1 produced by hybridoma (FERM ABP-10277).

60. Anti-ovomucoid monoclonal antibody PDOM2 produced by hybridoma (FERM ABP-10278).

61. A method for detecting flour allergens, wherein an anti-flour gliadin monoclonal antibody recognizing a native flour gliadin and a flour gliadin solubilized with a denaturant is used.

62. The method for detecting flour allergens, wherein 2 types of anti-flour gliadin monoclonal antibodies recognizing a native flour gliadin and a flour gliadin solubilized with a denaturant, and recognizing different epitopes are used in combination.

63. The method for detecting flour allergens according to claim 61 or 62, wherein the anti-flour gliadin monoclonal antibody recognizes a native flour gliadin, a reduced-carboxymethylated flour gliadin, a flour gliadin solubilized with 0.1 M acetate, a flour gliadin solubilized with 70% ethanol, and a flour gliadin solubilized with a denaturant.

64. The method for detecting flour allergens according to claims 61 to 63, wherein the anti-flour gliadin monoclonal antibody is the anti-flour gliadin monoclonal antibody PGL1 produced by hybridoma (FERM ABP-10267) and/or the anti-flour gliadin monoclonal antibody PGL2 produced by hybridoma (FERM ABP-10268).

65. The method for detecting flour allergens according to any one of claims 61 to 64, wherein the native flour gliadin, the reduced-carboxymethylated flour gliadin, the flour gliadin solubilized with 0.1 M acetate, the flour gliadin solubilized with 70% ethanol and the flour gliadin solubilized with a denaturant in foods can be analyzed qualitatively and quantitatively even at a concentration in a range of 10 to 100 ppb by sandwich ELISA.

66. A kit for detecting flour allergens comprising an anti-flour gliadin monoclonal antibody recognizing a native flour gliadin and a flour gliadin solubilized with a denaturant.

67. A kit for detecting flour allergens comprising 2 types of anti-flour gliadin monoclonal antibodies recognizing a native flour gliadin and a flour gliadin solubilized with a denaturant, and recognizing different epitopes.

68. The kit for detecting flour allergens according to claim 66 or 67, wherein the anti-flour gliadin monoclonal antibody recognizes a native flour gliadin, a reduced-carboxymethylated flour gliadin, a flour gliadin solubilized with 0.1 M acetate, a flour gliadin solubilized with 70% ethanol and a flour gliadin solubilized with a denaturant.

69. The kit for detecting flour allergens according to any one of claims 66 to 68, wherein the anti-flour gliadin

monoclonal antibody is the anti-flour gliadin monoclonal antibody PGL1 produced by hybridoma (FERM ABP-10267) and/or the anti-flour gliadin monoclonal antibody PGL2 produced by hybridoma (FERM ABP-10268).

70. The kit for detecting flour allergens according to any one of claims 66 to 69, wherein at least one of the 2 types of monoclonal antibodies recognizing different epitopes is a monoclonal antibody labeled with gold colloid used for immunochromatography.

71. Anti-flour gliadin monoclonal antibody PGL1 produced by hybridoma (FERM ABP-10267).

72. Anti-flour gliadin monoclonal antibody PGL2 produced by hybridoma (FERM ABP-10268).

73. A method for detecting buckwheat allergens, wherein an anti-buckwheat crude protein monoclonal antibody recognizing a native buckwheat crude protein and a heat-denatured buckwheat crude protein is used.

74. A method for detecting buckwheat allergens, wherein 2 types of anti-buckwheat crude protein monoclonal antibodies recognizing a native buckwheat crude protein and a heat-denatured buckwheat crude protein, and recognizing different epitopes are used in combination.

75. The method for detecting buckwheat allergens

according to claim 73 or 74, wherein the anti-buckwheat crude protein monoclonal antibody recognizes a 24Da protein and a heat-denatured buckwheat crude protein, or an anti-buckwheat crude protein monoclonal antibody recognizing a 76kDa protein and a native buckwheat crude protein.

76. The method for detecting buckwheat allergens according to any one of claims 73 to 75, wherein the anti-buckwheat crude protein monoclonal antibody is the anti-24kDa protein monoclonal antibody PBW1 produced by hybridoma (FERM ABP-10272) and/or the anti-76kDa protein monoclonal antibody PBW2 produced by hybridoma (FERM ABP-10273), and/or the anti-76kDa protein monoclonal antibody PBW3 produced by hybridoma (FERM ABP-10274).

77. The method for detecting buckwheat allergens according to any one of claims 73 to 76, wherein the native buckwheat crude protein and the heat-denatured buckwheat crude protein can be analyzed qualitatively and quantitatively even at a concentration in a range of 10 to 1000 ppb by sandwich ELISA.

78. The method for detecting buckwheat allergens according to any one of claims 73 to 77, wherein the heat-denatured buckwheat crude protein are extracted with the use of urea and 2-mercaptoethanol from a sample.

79. A kit for detecting buckwheat allergens, comprising

an anti-buckwheat crude protein monoclonal antibody recognizing a native buckwheat crude protein and a heat-denatured buckwheat crude protein.

80. A kit for detecting buckwheat allergens, comprising 2 types of anti-buckwheat crude protein monoclonal antibodies recognizing a native buckwheat crude protein and a heat-denatured buckwheat crude protein, and recognizing different epitopes.

81. The kit for detecting buckwheat allergens according to claim 79 or 80, wherein the anti-buckwheat crude protein monoclonal antibody recognizes a 24Da protein and a heat-denatured buckwheat crude protein or recognizes a 76kDa protein and a native buckwheat crude protein.

82. The kit for detecting buckwheat allergens according to any one of claims 79 to 81, wherein the anti-buckwheat crude protein monoclonal antibody is the anti-24kDa protein monoclonal antibody PBW1 produced by hybridoma (FERM ABP-10272) and/or the anti-76kDa protein monoclonal antibody PBW2 produced by hybridoma (FERM ABP-10273) and/or the anti-76kDa protein monoclonal antibody PBW3 produced by hybridoma (FERM ABP-10274).

83. The kit for detecting buckwheat allergens according to any one of claims 79 to 82, wherein at least one of the 2 types of monoclonal antibodies recognizing different epitopes is a monoclonal antibody labeled with gold

colloid used for immunochromatography.

84. The kit for detecting buckwheat allergens according to any one of claims 79 to 83, comprising urea and 2-mercaptoethanol as an agent for extracting buckwheat crude proteins from a test substance.

85. Anti-24kDa protein monoclonal antibody PBW1 produced by hybridoma (FERM ABP-10272).

86. Anti-76kDa protein monoclonal antibody PBW2 produced by hybridoma (FERM ABP-10273).

87. Anti-76kDa protein monoclonal antibody PBW3 produced by hybridoma (FERM ABP-10274).

88. A method for detecting peanut allergens, wherein an anti-Ara h1 protein monoclonal antibody recognizing a native peanut Ara h1 protein and a heat-denatured peanut Ara h1 protein is used.

89. A method for detecting peanut allergens, wherein 2 types of anti-Ara h1 protein monoclonal antibodies recognizing a native peanut Ara h1 protein and a heat-denatured peanut Ara h1 protein, and recognizing different epitopes are used in combination.

90. The method for detecting peanut allergens according to claim 88 or 89, wherein the anti-Ara h1 protein

monoclonal antibody recognizes a native Ara h1 protein and a native peanut crude protein, and/or an urea-treated Ara h1 protein and an urea-treated peanut crude protein.

91. The method for detecting peanut allergens according to any one of claims 88 to 90, wherein the anti-peanut Ara h1 protein monoclonal antibody is the anti-native Ara h1 protein monoclonal antibody PAh1-1 produced by hybridoma (FERM ABP-10269), and/or the anti-native Ara h1 protein monoclonal antibody PAh1-2 produced by hybridoma (FERM ABP-10270), and/or the anti-heat-denatured Ara h1 protein monoclonal antibody PAh1-3 produced by hybridoma (FERM ABP-10271).

92. The method for detecting peanut allergens according to any one of claims 88 to 91, wherein the native peanut Ara h1 protein and the heat-denatured peanut Ara h1 protein can be analyzed qualitatively and quantitatively even at a concentration in a range of 10 to 1000 ppb by sandwich ELISA.

93. The method for detecting peanut allergens according to any one of claims 88 to 92, wherein the heat-denatured peanut Ara h1 protein is extracted with the use of urea and 2-mercaptoethanol from a sample.

94. A kit for detecting peanut allergens comprising an anti-peanut Ara h1 protein monoclonal antibody recognizing a native peanut Ara h1 protein and a

heat-denatured peanut Ara h1 protein.

95. A kit for detecting peanut allergens comprising 2 types of anti-peanut Ara h1 protein monoclonal antibodies recognizing native peanut Ara h1 proteins and heat-denatured peanut Ara h1 proteins, and recognizing different epitopes.

96. The kit for detecting peanut allergens according to claim 94 or 95, wherein the anti-Ara h1 protein monoclonal antibody recognizes a native Ara h1 proteins and a native peanut crude protein, and/or an urea-treated Ara h1 protein and an urea-treated peanut crude proteins.

97. The kit for detecting peanut allergens according to any one of claims 94 to 96, wherein the anti-peanut Ara h1 protein monoclonal antibody is the anti-native Ara h1 protein monoclonal antibody PAh1-1 produced by hybridoma (FERM ABP-10269), and/or the anti-native Ara h1 protein monoclonal antibody PAh1-2 produced by hybridoma (FERM ABP-10270), and/or the anti-native Ara h1 protein monoclonal antibody PAh1-3 produced by hybridoma (FERM ABP-10271).

98. The kit for detecting peanut allergens according to any one of claims 94 to 97, wherein at least one of the 2 types of monoclonal antibodies recognizing different epitopes is a monoclonal antibody labeled with gold colloid used for immunochromatography.

99. The kit for detecting peanut allergens according to any one of claims 94 to 98, comprising urea and 2-mercaptoethanol as an agent for extracting whey proteins from a test substance.

100. Anti-native Ara h1 protein monoclonal antibody PAh1-1 produced by hybridoma (FERM ABP-10269).

101. Anti-native Ara h1 protein monoclonal antibody PAh1-2 produced by hybridoma (FERM ABP-10270).

102. Anti-heat-denatured Ara h1 protein monoclonal antibody PAh1-3 produced by hybridoma (FERM ABP-10271).